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Title of invention	**Agent for cancer gene therapy**
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Purpose	
Summary	 **Configurations,** including gene therapy agent for cancer vaccine was introduced into the tumor cell and tumor cell effector cytokine genes cytokine genes.

Claims

- Claim 1, including a gene therapy agent for cancer vaccine was introduced into the tumor cell and tumor cell effector cytokine genes cytokine genes.

[2] claim of claim 1, a gene therapy agent is involved in lymphocyte effector cells destroy cancer cells.

Claim 3 tumor-infiltrating lymphocytes lymphocytes (TIL), lymphokine activated killer cells (LAK), or cytotoxic T cells (CTL) is a therapeutic agent of claim 2 gene.

Claim 4 melanoma tumor cells, renal cell carcinoma, breast carcinoma cells, squamous cell carcinoma, adenocarcinoma, transitional cell carcinoma, sarcoma, or gliomas (Gurukoma) is, a gene therapy agent according to claim 1 .

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According to claim 5 cytokine gene introduced into effector cells, interleukin (IL) -2 gene therapy agent according to claim 1 gene.
According to claim 6 cytokine genes introduced into tumor cells, granulocyte-macrophage colony-stimulating factor (GM-CSF) gene, interleukin (IL) -2 gene therapy of claim 1 wherein the at least one gene agents.
Claim 7 of the gene therapy agent according to claim 1 by adenovirus vector was introduced into the effector cell cytokine genes.
Claim 8 of the gene therapy agent according to claim 1 was introduced into tumor cells by retroviral cytokine genes.

DETAILED DESCRIPTION OF THE INVENTION

[0001]
[Industrial Field of the invention relates to gene therapy agent for cancer, more particularly, an agent for gene therapy of cancer, including tumor cell vaccine was introduced into the tumor cells and effector cytokine genes cytokine genes.

[0002]
[ART cytokine that exerts antitumor effects are divided into the following three main mechanisms of its action. That is, Tumor necrosis factor ■ (TNF)-α, β, interferon (IFN)-α, β, γ, interleukin (IL) -1 and so on, no cancer cells in vitro and in direct antiproliferative shows cytotoxic activity, Interleukin ■ (IL) -2, 4,6, 7, 8, 9, 10, 11, shows the antitumor effect indirectly through the body's immune effector mechanisms, such as 12, ■ IL-3, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), stem cell factor (SCF) of living with other anticancer therapies such as which has been used to treat or prevent side effects.

[0003] In addition, cancer treatments have been attempted in recent years by the introduction of cytokine genes. One of these lymphocyte cytokine genes, such as lymphokine activated killer cells (LAK), cytotoxic T cells (CTL), tumor-infiltrating lymphocytes (TIL) and introduced, which is secreted from the cell site deployment Enhancing the passive immunity to the activation of autocrine or paracrine lymphocytes by The Chi, TIL have direct antitumor IFN or TNF in an attempt to enhance the antitumor activity in tumor site by introducing a gene have been made (Nishihara et al., Cancer Res., 48, 4730, 1988, Miyatakeet al., J. Natl. Cancer Inst., 82, 217, 1990, Itoh et al., Jpn. J. Cancer Res. , 82, 1203, 1991). TIL cytokine gene transfer into the retrovirus has been used so far is very poor transduction efficiency, this approach has become an obstacle to.

[0004] Another is used as a tumor vaccine to introduce cytokine genes into tumor cells by a retrovirus and is intended to induce a tumor-specific immune cells of the host. For example, Fearon et al IL-2 gene into mouse colon cancer, spontaneous remission and survival after transplantation into syngeneic mouse melanoma, the mouse IL-resistant tumor cell reimplantation of introducing non-2 gene have reported that a high degree of acquired immunity (Fearon et al., Cell, 60, 397, 1990). However, these are obtained eiter not always satisfactory grades for antitumor activity both in terms of metastasis suppression has not been examined and, what appears to be promising in cancer metastasis At present, not been reported.

[0005]
Problems to be Solved by the Invention] Accordingly, the purpose of the present invention is to solve the above problems, to exert anti-tumor activity achieved a higher cancer gene therapy is also effective in suppressing cancer metastasis and agents have to provide.

[0006]
[MEANS FOR SOLVING PROBLEMS] The present inventors have conducted extensive studies on the results of the above challenges, when combined with tumor vaccine was introduced into tumor cells by effector cells and cytokine genes cytokine genes, We found that metastasis suppression is enhanced antitumor effects and systemic immunity is induced efficiently and completed the invention.

[0007] The present invention provides an agent for gene therapy of cancer, including tumor cell vaccine was introduced into the tumor cells and effector cytokine genes cytokine genes. Cancer gene therapy and referred to the invention, which contemplates the treatment of cancer metastasis and inhibition of both anti-tumor activity of cytokine genes.

[0008] The following describes in detail the invention. Cytokine genes and used in the invention, granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL) -2, IL-3, IL-4, IL-6, IL-7, IL- 10, IL-12, IL-13, IL-15, interleukin-1α (IL-1α), a Intaroikinreseputa antagonist (IL-1RA), tumor necrosis factor (TNF)-α, lymphotoxin (LT) -β, granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), interferon (IFN)-γ, Macro Phage migration inhibitory factor (MIF), leukemia inhibitory factor (LIF), T cell activation costimulatory factor B7 (CD80) and B7-2 (CD86), kit ligand, oncostatin M gene coding for the various cytokines, such as says. Various cytokines have previously been cloned cDNA [human GM-CSF cDNA for Wong et al., Science, 228, 810-815 (1985), for the human IL-2 cDNA Taniguchi et al., Nature, 302, 305-310 (1983), human IFN-γ cDNA for Gray et al., Nature, 298, 859-863 (1982)]. Cytokine genes used in the invention, obtained from cells isolated cDNA using known techniques, even more polymerase chain reactions and literature information that is disclosed above and (PCR) method, etc. those which may be chemically

synthesized according to minimize immune rejection, and to increase the therapeutic effect of human origin is desirable.
[0009] In the present invention and the effector cells responsible for the final stage of the destruction of cancer cells, like cell population directly involved in the destruction, specifically in tumor-infiltrating lymphocytes (TIL), lymphokine activated killer cells (LAK), cytotoxic T cells (CTL) and other means. Cytokine gene transduction into effector cells, these can be very efficient by using the adenovirus vector. An adenovirus vector used here, including the insertion site for the cytokine genes, but are not particularly limited as long as it can express the effector cytokines were introduced in cells, derived from human adenovirus type 5 Adex1 [Saito , I. et al., J. Viol., 54, 711-719 (1985)] is preferably used.
[0010] In contrast, tumor vaccines referred to in the present invention, retroviral gene isolated by these cytokines (culture) is introduced into tumor cells, which make X-ray irradiation does not inhibit the production of cytokines which only halted growth. Into a host by administering tumor vaccines can induce tumor-specific immune cells of the host.
[0011] The retrovirus vector used here, including the insertion site for the cytokine genes, but are not particularly limited as long as it can express the cytokines tumor cells were introduced, for example, the table of special 6-503968 is disclosed in JP, MFG, α-SCG, PLJ, pEm and the like. Cytokine gene was inserted into retroviral vectors, plasmids and to mix with the neomycin resistance gene as a marker to select the desired gene is introduced that, Ψ2, Ψ-Am, ΨCRIP, ΨCRE [Danos et al., PNAS , 85, 6460-6464 (1988) introduced by co-precipitation of calcium, such as packaging cells (cotransfection). This drug further cultured in the presence of G418, cells were harvested by colonies come alive, the introduction of the target gene You can only cells that are harvested. Using these cell culture supernatant of NIH3T3 mouse fibroblasts and then, B16 and various tumor cells were infected with mouse melanoma cells, and ultimately a stable transgenic cells were introduced into the chromosome of the cell, Sazanhai can be confirmed by Buridaizeshon. The amount of cytokines secreted by infected cells, ELISA assays can be measured by immunological others.
[0012] Next, the introduction of cytokine genes tumor cells, usually after 10,000 ~ 150,000 rad of X-rays, which are used as tumor vaccines. Most of this tumor cell, or kidney cancer cells are melanoma cells, other tumor cells, such as breast cancer cells, squamous cell carcinoma, adenocarcinoma, transitional cell carcinoma, sarcoma, gliomas (gliomas), and also be used possible. Tumor vaccine cells and effector transgenic cytokines were prepared to make the above may be used directly as pharmaceutical compositions and excipient pharmaceutically acceptable solutions, suspensions, pharmaceutical forms such as gels[Ε,Shi can be administered.
[0013] As the dosage form comprises a gene therapy agent, an intravenous general, in addition to intra-arterial and systemic administration for the original cancer lesion, corresponding to the expected site of metastasis or cancer types, local injection, topical administration can be performed, such as oral administration. In addition, the administration of gene therapeutic agent for Getting the invention, catheter technology, transgenic technology can also take the form given in combination with surgery or other. The dose of the gene therapy agent of the invention, age, sex, symptoms, route of administration, number of doses may vary depending on dosage form, typically in adults with the weight of the cytokine genes per day, about 0.1 ~ 100 mg in the range of be appropriate.
[0014]
[Effect of the Invention According to the invention, human or mouse, monkey, dog, cat, horse, give it possible for pigs and other animals high anti-tumor agents, gene therapy is also effective in suppressing cancer metastasis and is provided, useful in the treatment of cancer micrometastases.
[0015]
EXAMPLES described in more detail by following examples of the invention. These examples are for illustrative purpose, does not limit the scope of the invention.
Reference Example 1] Mouse IL-2, mouse GM-CSF, and mouse IFN-γ cDNA of the mRNA of mouse spleen lymphocytes using RT-PCR (Reverse transcripiton-polymerase chain reaction) was prepared by the method. Upon PCR to mouse IL-2 for the following primer # 479, # 480, mouse GM-CSF for the following primer # 477, # 478, mouse IFN-γ for the following primers shown in # 485, # 486 was used, respectively.
[0016] Primers (# 479): CCGAATTCTAGACACC ATG TAC AGC ATG CAG CTC GCA TCC TGT G primer (# 480): C TGT CAA AGC ATC ATC TCA ACA A GC CCT CAA TAA GGATCC CC primer (# 477): CCGAATTCTAGACACC ATG TGG CTG CAG AAT TTA CTT TTC CTG GGC primer (# 478): C CCC TTT GAA TGC AAA AAA CCA AGC CAA AAA TGA GGATCC GG primer (# 485): CC GAA TTC TAGA CACC ATG AAC GCT ACA CAC TGC ATC TTG GC primer (# 486): C AGG AAG CGG AAA AGG AGT CGC TGC TGA GGATCCGG] [0017] Example 1 effector cell cytokine gene (TIL/IL-2) Preparation of (1) Preparation of TIL derived from murine B16F10 preparation of TIL, Alexander, RB et al., J. Immunol., 145, 1615-1620 (1990), Matis, LA et al., Methods Enzymol., 150 , 342-351 (1987), Livingstone, A. et al., Methods Enzymol., 150, 325-333 (1987) went to make the following improvements to the method described. 6 week-old female 10 C57BL / mouse 6 (Charles River Japan from Canada) were transplanted into murine B16 (ATCC CRL6322) is a strain of the highly metastatic B16F10 (Whitehead Institute Dr.Glenn Dranoff obtained from) the fresh tumor masses complete culture medium (CM) 5 ℃ 4 while in × 7 10 cells / ml suspension. The CM

was heat-inactivated 10% fetal calf serum, 2mM L-glutamine, 5×10^{-5} M 2 - mercaptoethanol, 100U/ml penicillin, μg 100 / ml streptomycin, 0.5 μg / ml amphotericin B, 10mM 3 - (N-morpholino) propanesulfonic acid, 70U/ml and recombinant human IL-2 (Shionogiseiyaku (Co) obtained from) was added to the RPMI 1640. TIL against an equal volume of beads and CD-8-1-linked immunosorbent $\times 8$ 10 / ml mixed and incubated for 2 hours ℃ 4. TIL beads that are contaminated with the pellet with cold CM and washed three times, CM 1 in $\times 7$ 10 beads / ml suspension, were seeded into tissue culture plate wells 24, ℃ 37, 5% CO were incubated in $_2$. One day after culture, TIL isolated from the pelleted beads were removed. TIL 2 per well was separated into individual exposure $\times 5$ 10 (10,000 rad) tumor cells, and a number of exposure $\times 6$ 10 (3,000 rad) spleen cells were stimulated using a fine. repeated every 14 days from the date of in vitro stimulation 7. Aliquot of TIL when confluent, and in the new CM 2×5 10 cell / ml and resuspended.

[0018] (2) gene in mice by adenovirus-IL-2 TIL method for preparing a recombinant adenovirus to the introduction, Saito, I. et al., J. Viol., 54,711-719 (1985) variant of went through. That is, Saitomegarouirusuenhansa, β-actin chicken, mouse prepared in Reference Example 1 of IL-2 cDNA sequence, rabbit-β-Gurobinpori (A) expression unit comprising a signal sequence [Niwa, H. et al., J. Gene 108, 193-200 (1991)] a, E1A, E1B, you E3 gene and lack of 42kb of 31kb, including five cosmids Adenouirusutaipu pAdex1w (Hiromi Kanegae, Harada Shizuko, Izumi Saito, Bio-Manual Experimental Medicine 4,189-204, 1994, the company Sat sheep) of SwaI restriction site Expression was constructed by inserting Kosumidokasetto (Figure 1). For the expression and adenovirus Kosumidokasetto terminal DNA-protein complex (DNA-TPC) 293 cells (ATCC CRL1573) were cotransfected by the calcium coprecipitation. Expression cassette containing the recombinant virus was confirmed by digestion with appropriate restriction enzymes. Recombinant virus is then grown in 293 cells, the virus solution was stored at -80 ℃. Titer viral stock was determined by plaque assay on 293 cells. (1) were prepared in TIL to the adenovirus for in vitro infection, the culture medium 12 - well culture plates seeded with the exception of TIL cells μl 150 virus stock was added to each well. After ineubation for 1 hour ℃ 37, added to the growth medium, TIL 2 - cells were cultured for 3 days, transgenic mouse IL-2 TIL cells (TIL/IL-2) was obtained.

[0019] [Example 2 tumor vaccine (B16F10/IL-2 + GM-CSF vaccine) Preparation of (1), mouse IL-2, mouse GM-CSF retroviral preparation of high-titer recombinant retrovirus producing clones MFG (Dranoff , G. et al., Proc. Natl. Acad. Sci. USA, 90, 3539-3543, 1993) Two LTR (Long Terminal Repeat) containing EagI / BamHI fragment (5200bp), EagI / XbaI fragment (1000bp) mice were prepared in Reference Example 1 and IL-2, mouse GM-CSF cDNA in each of the XbaI / BamHI fragment T4DNA incorporated into three conneeted by ligase plasmid (Fig. 2). The resulting plasmid pPGKneo [H. Takeshima et al., Nature, 369, 556-559 (1994)] ΨCRIP calcium co-precipitation method [Danos et al , Proc. Natl. Acad. Sci. USA, 85, 6460 (1988)] is introduced into this G-418 GIBCO, 1mg/ml) were cultured in medium containing one week, cells were collected cone to colonize. Culture supernatants of these cells then polybrene (Sigma, 8μg/ml) presence, NIH3T3 mouse fibroblasts (ATCC CRL1658) were infected. Genomic DNA was isolated infected cells, the virus titer measured by Southern blotting, were evaluated as the number of copies of integrated provirus. Efficiency is typically introduced into NIH3T3, 1 proviral integration copy number per cell ranged from 3. In addition, cytokines secreted by infected cells, the medium containing 10ml 10-cm 1 × dish seeded cells after 48 hours ^{6} 10, ELISA (Endogen) was assayed by (Table 1).

[0020]

[Table 1]

cDNA titer (number of copies) mice	expression level of IL-2 2.0 6350 IU / ml mouse GM -CSF 2.0 13.5 ng / ml

[0021] (2) tumor vaccines (B16F10/IL-2 + GM-SCF vaccine) Preparation of (1) selected by the mouse IL-2, mouse murine GM-CSF-producing clones of each high-titer recombinant retrovirus B16 (ATCC CRL 6322) is a strain of the highly metastatic B16F10 (Whitehead Institute Dr.Glenn Dranoff obtained from) was introduced. The gene transfected cells, 10% fetal bovine serum and 2mM were maintained in Dulbecco's Eagle medium glutamine, trypsin / EDTA treated with, HITACHI MBR-1505R X-ray generator used, 10,000 rad X-rays was. Irradiated cells HBSS (Hank's Balanced Salt Solution) and washed twice, 5×6 10 cell / ml resuspended in HBSS at a concentration, tumor vaccines (B16F10/IL-2 + GM-CSF vaccine) was obtained.

Example 3 [0022] [effector cell cytokine gene (TIL / IFN-γ) Preparation of (1) derived from B16F10 murine TIL 1 Preparation Example (1) was prepared in the same manner as this.

(2) gene in mice by adenovirus-IFN-γ TIL method for preparing a recombinant adenovirus to the introduction, Saito, I. et al., J. Viol., 54,711-719 (1985) carried out by a variant of . That is, Saitomegarouirusuenhansa, β-actin chicken, mouse prepared in Reference Example 1 in IFN-γ cDNA sequence, rabbit-β-Gurobinpori (A) expression unit comprising a signal sequence [Niwa, H. et al., J. Gene 108, 193-200 (1991)] a, E1A, E1B, E3 and the lack of a 42kb gene is a 31kb cosmid containing 5 Adenouirusutaipu pAdex1cw (Hiromi Kanegae, Harada Shizuko, Izumi Saito, Experimental Medicine, Bio-Manual 4,189-204, 1994, the company Sat sheep) for

expression of Kosumidokasetto ClaI was constructed by inserting restriction site (Fig. 1). For the expression and adenovirus Kosumidokasetto terminal DNA-protein complex (DNA-TPC) 293 cells (ATCC CRL1573) were cotransfected by the calcium coprecipitation. Expression cassette containing the recombinant virus was confirmed by digestion with appropriate restriction enzymes. Recombinant virus is then grown in 293 cells, the virus solution was stored at -80 ℃. Titer viral stock was determined by plaque assay on 293 cells. (1) were prepared in TIL to the adenovirus for in vitro infection, the culture medium 12 - well culture plates seeded with the exception of TIL cells μl 150 virus stock was added to each well. After incubation for 1 hour ℃ 37, added to the growth medium, TIL 2 - cells were cultured for 3 days, transgenic mouse IFN-γ TIL cells (TIL / IFN-γ) was obtained.

[0023] [Example 4 tumor vaccine (B16F10/GM-CSF vaccine) Preparation of (1), mouse GM-CSF 2 EXAMPLE Preparation of high-titer recombinant retrovirus producing clones (1) was prepared in a similar manner.

(2) cancer vaccine (B16F10/GM-SCF vaccine) Preparation of (1) selected by the mouse GM-CSF recombinant murine retrovirus clones producing high titer B16 (ATCC CRL 6322) is a strain of the highly metastatic B16F10 (obtained from WhiteheadInstitute Dr.Glenn Dranoff) was introduced. The gene transfected cells, 10% fetal bovine serum and 2mM were maintained in Dulbecco's Eagle medium glutamine, trypsin / EDTA treated with, HITACHI MBR-1505R X-ray generator used, 10,000 rad X-rays was. Irradiated cells were washed twice in HBSS, 5 × ^{6} 10 cell / ml were resuspended at a concentration of HBSS, tumor vaccines (B16F10/GM-CSF vaccine) was obtained.

[0024] [Example 5 effector cell cytokine gene (TIL/IL-2, TIL / IFN-γ) Preparation of (1) Colon cancer-derived mouse colon preparation of TIL 26 TIL preparations, Alexander, RB et al ., J. Immunol., 145, 1615-1620 (1990), Matis, LA et al., Methods Enzymol., 150, 342-351 (1987), Livingstone, A et al., Methods Enzymol., 150, 325-333 (1987) was to make the following improvements to the method described. 6 female weeks old 10 BALB / C mice (Charles RiverJapan purchased from), colon cancer mice implanted with Colon culture medium completely tumor mass of fresh 26 (CM) 5 in ℃ 4 in × ^{7} 10 cells / ml suspension suspended. The CM was heat-inactivated 10% fetal calf serum, 2mM L-glutamine, 5 × 10^{-5} M 2 - mercaptoethanol, 100U/ml penicillin, μg 100 / ml streptomycin, 0.5 μg / ml amphotericin B, 10mM 3 - (N-morpholino) propanesulfonic acid, 70U/ml and recombinant human IL-2 (Shionogiseiyaku (Co) obtained from) was added to the RPMI 1640. TIL against an equal volume of beads and CD-8-1-linked immunosorbent × ^{8} 10 / ml mixed and incubated for 2 hours ℃ 4. TIL beads that are contaminated with the pellet with cold CM and washed three times, CM 1 in × ^{7} 10 beads / ml suspension, were seeded into tissue culture plate wells 24, ℃ 37, 5% CO were incubated in $_{2}$. One day after culture, TIL isolated from the pelleted beads were removed. TIL 2 per well was separated into individual exposure × ^{5} 10 (10,000 rad) tumor cells, and a number of exposure × ^{6} 10 (3,000 rad) spleen cells were stimulated using a fine. repeated every 14 days from the date of in vitro stimulation 7. Aliquot of TIL when confluent, and in the new CM 2 × ^{5} 10 cell / ml resuspension Was.

[0025] (2) gene in mice by adenovirus-IL-2 TIL 1 Introduction to the Example (2) by the same method as above (1) mouse TIL were prepared in Reference Example 1 to prepare an IL-2cDNA introduced, transgenic mice IL-2 TIL cells (TIL/IL-2) was obtained.

(3) gene in mice by adenovirus-IFN-γ TIL 3 Introduction to the Example (2) by the same method as above (1) prepared in Reference Example 1 was prepared by TIL to mouse IFN-introduced γcDNA, transgenic mouse IFN-γ TIL cells (TIL / IFN-γ) was obtained.

[0026] [Example 6 tumor vaccine (Colon26/IL-2 vaccine) Preparation of (1), mouse IL-2 producing clones prepared in Example 2 high-titer recombinant retrovirus (1) was prepared in a similar manner.

(2) cancer vaccine (Colon26/IL-2 vaccine) Preparation of (1) selected by the mouse IL-colon cancer clones producing high-titer recombinant retrovirus was introduced into 2 Colon26. The gene transfected cells, 10% fetal bovine serum and 2mM glutamine were maintained in RPM11640 medium, HITACHI MBR-1505 R X-ray generator used, 10,000 rad of X-rays. Irradiated cells were washed twice in HBSS, 5 × ^{6} 10 cell / ml were resuspended at a concentration of HBSS, tumor vaccines (Colon26/IL-2 vaccine) was obtained.

[0027] Test Example 1] based on efficacy of pulmonary metastases (1)

6 week-old female 10 C57BL / mouse 6 (Charles River Japan from Canada), 4 × ^{5} 10 B16F10 murine number of cells were inoculated into the tail vein and allowed to induce lung metastases. 2 TIL days alone, or prepared in Example 1 was administered intravenously TIL/IL-2 4 × the number ^{6} 10 [E / T ratio = 10: E / T effector cells (TIL/IL-2) Number / tumor cells (B16F10) represents the number. Example 2 was prepared by B16F10/IL-2 + GM-CSF 5 At the same time the vaccine was administered subcutaneously ^{5} × 10. Counting the number of lung metastatic nodules caused more dissected 16 days after induction of lung metastasis. TIL alone as a comparison or what do TIL/IL-2 were also tested for dose vaccination alone. Table 2 and Figure 3 shows the results.

[0028]

TABLE 2

[0029] and TIL/IL-2 B16F10/IL-2 + GM-CSF was administered in combination with the vaccine group showed a significant decrease in the number of metastatic nodules in particular, with the maximum inhibitory effect on lung cancer metastasis was confirmed.
[0030] Test Example 2] based on efficacy in metastatic (2)
6 week-old female 10 C57BL / mouse 6 (Charles River Japan from Canada), 3×5 10 B16F10 murine number of cells were inoculated into the tail vein and allowed to induce lung metastases. 2 TIL days alone, prepared in Example 3 or TIL / IFN-γ 4.5 to Each was administered intravenously $\times 6$ 10 (E / T ratio = 15). Example 4 was prepared at the same time the vaccine B16F10/GM-CSF 5×10^5 was administered subcutaneously. Counting the number of metastatic nodules caused lung dissected from 16 days after induction of lung metastasis. As a comparison alone TIL, TIL / IFN-treatment only those vaccinated do γ, were also tested for only B16F10/GM-CSF or vaccination. Table 3 and Figure 4 shows the results.
[0031]
TABLE 3

[0032] The TIL / IFN-γ B16F10/GM-CSF groups and administered with a combination vaccine showed a significant decrease in the number of metastatic nodules in particular, can be confirmed that the maximum inhibitory effect on lung cancer metastasis or.
[0033] Test Example 3] based on efficacy of pulmonary metastases (3)
6 week-old female 10 BALB / C mice (Charles River Japan from Canada), 2×4 10 Colon number of murine colon cancer 26 cells were inoculated into the tail vein and allowed to induce lung metastases. 2 TIL days alone, or prepared in Example 5 or TIL/IL-2 TIL / IFN-1 was administered intravenously to each γ × 6 10 (E / T ratio = 50). Example 6 was prepared by the vaccine at the same time Colon26/IL-2 5×10^5 was administered subcutaneously. Counting the number of metastatic nodules in the lungs resulting than 18 days after the autopsy to induce lung metastasis. Compared with With TIL alone, TIL / IFN-treatment only in those vaccinated do γ, were also tested for only Colon26/IL-2 or vaccination. Table 4 and Figure 5 shows the results.
[0046]
TABLE 4

[0035] TIL / IFN-γ Colon26/IL-2 and vaccines, and TIL/IL-2 or group Colon26/IL-2 vaccine was administered in combination showed a significant decrease in the number of metastatic nodules, especially lung was confirmed that the maximum suppression of cancer metastasis.